Amendments to the Specification:

In compliance with 37 C.F.R. § 1.823(a), please insert the attached paper copy of the "Sequence Listing" after the last page of the above-identified application.

Please replace paragraph [0016] on page 4 with the following amended paragraph:

[0016] The modified GPCRs of the present invention include GPCRs comprising a NPXXY motif (SEQ. ID NO.: 82), a putative site of palmitoylation, and a modified carboxyl terminal tail. The carboxyl terminal tail may include one or more additions, substitutions, mutations, or deletions of amino acid residues such that the carboxyl-terminal tail comprises one or more sites of phosphorylation, preferably clusters of phosphorylation sites. The phosphorylation sites are positioned such that they are approximately 15 to 35 (preferably 15 to 25) amino acid residues downstream of the putative site of palmitoylation of the modified GPCR. The modified carboxyl terminal tail may be modified by discrete point mutations.

Please replace paragraph [0018] beginning on page 4 with the following amended paragraph:

[0018] In a further aspect, the modified ~~GPGRs~~ GPCRs of the present invention include GPCRs comprising a NPXXY motif (SEQ. ID NO.: 82) and a carboxyl terminal tail. The carboxyl terminal tail comprises a putative site of palmitoylation and one or more clusters of phosphorylation sites. The carboxyl-terminal tail may comprise a retained portion of a carboxyl-terminus region of a first GPCR fused to a portion of a carboxyl-terminus from a second GPCR. The second GPCR comprises the one or more sites of phosphorylation, preferably clusters of phosphorylation sites. The second GPCR further comprises a putative site of palmitoylation approximately 10 to 25 amino acid residues, preferably approximately 15 to 20 amino acid residues, downstream of a NPXXY motif (SEQ. ID NO.: 82).

Please replace paragraph [0019] beginning on page 5 with the following amended paragraph:

[0019] In an additional aspect, the modified GPCRs of the present invention also may include GPCRs comprising a NPXXY motif (SEQ. ID NO.: 82) and a carboxyl-terminal tail. The carboxyl terminal tail comprises a palmitoylated cysteine residue and one or more sites of phosphorylation, preferably clusters of phosphorylation sites. The carboxyl terminal tail of the modified receptor may comprise a retained

portion of a ~~carboyl-terminus~~ carboxyl-terminus region of a first GPCR fused to a portion of a carboxyl-terminus from a second GPCR. The second GPCR comprises the one or more sites of phosphorylation, preferably clusters of phosphorylation sites. The retained portion of the first GPCR and the second GPCR are fused at an amino acid residue adjacent to the palmitoylated cysteine residue.

Please replace paragraph [0093] beginning on page 21 with the following amended paragraph:

[0093] "Carboxyl-terminal tail" means the carboxyl-terminal tail of a GPCR. The carboxyl-terminal tail of many GPCRs begins shortly after the conserved NPXXY motif (SEQ. ID NO.: 82) that marks the end of the seventh transmembrane domain (i.e. what follows the NPXXY motif (SEQ. ID NO.: 82) is the carboxyl-terminal tail of the GPCR). The carboxyl-terminal tail may be relatively long (approximately tens to hundreds of amino acids), relatively short (approximately tens of amino acids), or virtually non-existent (less than approximately ten amino acids). As used herein, "carboxyl-terminal tail" shall mean all three variants (whether relatively long, relatively short, or virtually non-existent).

Please replace paragraph [0101] beginning on page 24 with the following amended paragraph:

[0101] "Putative site of palmitoylation" means an expected site of palmitate addition, preferably a cysteine residue. In the GPCRs used in the present invention, the putative site of palmitoylation is preferably 10 to 25, preferably 15 to 20, amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82).

Please replace paragraph [0103] beginning on page 24 with the following amended paragraph:

[0103] "NPXXY motif" (SEQ. ID NO.: 82) means a conserved amino acid motif that marks the end of the seventh transmembrane domain. The conserved amino acid motif begins with asparagine and proline followed by two unspecified amino acids and then a tyrosine. The two unspecified amino acids may vary among GPCRs but the overall NPXXY motif (SEQ. ID NO.: 82) is conserved.

Please replace paragraph [0132] beginning on page 32 with the following amended paragraph:

[0132] The present invention is related to modified GPCRs. Modified GPCRs of the present invention may comprise one or more modifications in their carboxyl-terminal tail. These modifications may comprise inserting one or more sites of phosphorylation, preferably clusters of phosphorylation sites, within certain regions of the carboxyl-terminal tail. As such, the carboxyl-terminal tail may be modified in whole or in part. The carboxyl-terminal tail of many GPCRs begins shortly after a conserved NPXXY motif (SEQ. ID NO.: 82) that marks the end of the seventh transmembrane domain (i.e. what follows the NPXXY motif (SEQ. ID NO.: 82) is the carboxyl-terminal tail of the GPCR). The carboxyl-terminal tail of many GPCRs comprises a putative site of palmitoylation approximately 10 to 25 amino acid residues, preferably 15 to 20 amino acid residues, downstream of the NPXXY motif (SEQ. ID NO.: 82). This site is typically one or more cysteine residues. The carboxyl-terminal tail of a GPCR may be relatively long, relatively short, or virtually non-existent. The present inventors have determined that the carboxyl-terminal tail of a GPCR determines the affinity of arrestin binding.

Please replace paragraph [0134] beginning on page 33 with the following amended paragraph:

[0134] GPCRs that form stable complexes with arrestin comprise one or more sites of phosphorylation, preferably clusters of phosphorylation sites. In addition to the presence of the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, it has been discovered that the sites must be properly positioned within the carboxyl-terminal tail to promote formation of a stable GPCR/arrestin complex. To promote formation of a stable GPCR/arrestin complex, the one or more sites of phosphorylation, preferably one or more clusters of phosphorylation, may be approximately 15 to 35 (preferably 15 to 25) amino acid residues downstream of a putative site of palmitoylation of the GPCR. In addition, the one or more sites of phosphorylation, preferably one or more clusters of phosphorylation, may be approximately 20 to 55 (preferably 30 to 45) amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82) of the GPCR. GPCRs containing one or more sites of phosphorylation, preferably clusters of phosphorylation sites, properly positioned are typically Class B receptors.

Please replace paragraph [0135] beginning on page 34 with the following amended paragraph:

[0135] By way of example, it has been discovered that the V2R receptor comprises a cluster of phosphorylation sites (SSS) that promotes formation of a stable GPCR/arrestin complex at 19 amino acid residues downstream of the putative site of palmitoylation and 36 amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82). The NTR-2 receptor comprises a cluster of phosphorylation sites (STS) that promotes formation of a stable GPCR/arrestin complex at 26 amino acid residues downstream of the putative site of palmitoylation and 45 amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82). The oxytocin receptor (OTR) receptor comprises two clusters of phosphorylation sites (SSLST and STLS) that promote formation of a stable GPCR/arrestin complex, one at 20 amino acid residues downstream of the putative site of palmitoylation and the other at 29 amino acid residues downstream of the putative site of palmitoylation, and one at 38 amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82) and the other at 47 amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82), respectively. The substance P receptor (SPR, also known as the neurokinin-1 receptor) comprises a cluster of phosphorylation sites (TTIST) that promotes formation of a stable GPCR/arrestin complex at 32 amino acid residues downstream of the putative site of palmitoylation and 50 amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82).

Please replace paragraph [0138] beginning on page 35 with the following amended paragraph:

[0138] The modified GPCRs of the present invention include GPCRs containing a NPXXY motif (SEQ. ID NO.: 82), a putative site of palmitoylation approximately 10 to 25 amino acid residues (preferably 15 to 20 amino acids) downstream of the NPXXY motif (SEQ. ID NO.: 82), and a modified carboxyl-terminal tail. The modified carboxyl-terminal tail has one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites, such that the phosphorylation sites are approximately 15 to 35, preferably 15 to 25, amino acid residues downstream of the putative site of palmitoylation of the modified GPCR. The modified carboxyl-terminal tail may have one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites, such that the phosphorylation sites are approximately 20 to 55, preferably 30 to 45, amino acid residues downstream of the NPXXY (SEQ. ID NO.: 82) of the modified GPCR.

Please replace paragraph [0143] beginning on page 37 with the following amended paragraph:

[0143] Furthermore, to provide modified GPCRs of the present invention, a GPCR lacking properly positioned phosphorylation sites or with a lower or unknown affinity for arrestin may also have its carboxyl-terminal tail, in whole or in part, exchanged with that of a GPCR having properly positioned clusters of phosphorylation sites. The site of exchange may be after or including the conserved NPXXY motif (SEQ. ID NO.: 82). As an alternative, a putative site of palmitoylation of a GPCR may be identified at approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the conserved NPXXY motif (SEQ. ID NO.: 82), and the site of exchange may be after or including the palmitoylated cysteine(s). Preferably, the carboxyl-terminal tail of a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin is exchanged at an amino acid residue in close proximity to a putative site ~~a~~ of palmitoylation. More preferably, the carboxyl-terminal tail of a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin is exchanged at a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82), such that the palmitoylated cysteine residue is maintained. Exchanging in the preferred manner allows the clusters of phosphorylation sites to be reliably positioned properly within the carboxyl-terminal tail of the modified GPCR. The tails may be exchanged and the modified GPCRs may be constructed accordingly by manipulation of the nucleic acid sequence or the corresponding amino acid sequence.

Please replace paragraph [0144] beginning on page 38 with the following amended paragraph:

[0144] In a further alternative, the carboxyl-tail of a GPCR, for example a GPCR not containing the NPXXY motif (SEQ. ID NO.: 82), may be predicted from a hydrophobicity plot and the site of exchange may be selected accordingly. Based on a hydrophobicity plot, one of skill in the art may predict a site where it is expected that the GPCR may anchor in the membrane and then predict where to introduce a putative site of palmitoylation accordingly. Using this technique GPCRs having neither a NPXXY motif (SEQ. ID NO.: 82) nor a putative site of palmitoylation may be modified to create a point of reference (e.g. a putative site of palmitoylation). The introduced putative site of palmitoylation may then be used to position a tail exchange.

Please replace paragraph [0145] beginning on page 38 with the following amended paragraph:

[0145] The carboxyl-terminal tail used for the exchange may be from a second GPCR having one or more properly positioned clusters of phosphorylation sites and having a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif (SEQ. ID NO.: 82). The tail as identified may be exchanged, after or including the conserved NPXXY motif (SEQ. ID NO.: 82). As an alternative, a putative site of palmitoylation of a GPCR may be identified at approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the conserved NPXXY motif (SEQ. ID NO.: 82), and the tail may be exchanged after or including the palmitoylated cysteine(s). Preferably, the carboxyl-terminal tail of a GPCR having clusters of phosphorylation sites is exchanged at an amino acid residue in close proximity to a putative site of palmitoylation. More preferably, the carboxyl-terminal tail of a GPCR having clusters of phosphorylation sites is exchanged at a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82), such that the portion of the carboxyl-terminal tail containing the clusters of phosphorylation sites begins at the amino acid residue immediately downstream of the palmitoylated cysteine residue. Exchanging in the preferred manner allows the clusters of phosphorylation sites to be reliably positioned properly within the carboxyl-terminal tail of the modified GPCR. The carboxyl-terminal tail having clusters of phosphorylation sites used for the exchange may have a detectable molecule conjugated to the carboxyl-terminus. The tails may be exchanged and the modified GPCRs may be constructed accordingly by manipulation of the nucleic acid sequence or the corresponding amino acid sequence.

Please replace paragraph [0146] beginning on page 39 with the following amended paragraph:

[0146] In addition, the carboxyl-terminal tail portion used for the exchange may originate from a polypeptide synthesized to have an amino acid sequence corresponding to an amino acid sequence from a GPCR having one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites. The synthesized polypeptide may have a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif (SEQ. ID NO.: 82). The synthesized polypeptide may have one or more additions, substitutions, mutations, or deletions of amino acid residues that does not affect or alter the overall structure and function of the polypeptide.

Please replace paragraph [0147] beginning on page 39 with the following amended paragraph:

[0147] Furthermore, the carboxyl-terminal tail portion used for the exchange may originate from a naturally occurring polypeptide recognized to have an amino acid sequence corresponding to an amino acid sequence from a GPCR having one or more clusters of phosphorylation sites. The polypeptide may have a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif (SEQ. ID NO.: 82). The polypeptide may have one or more additions, substitutions, mutations, or deletions of amino acid residues that does not affect or alter the overall structure and function of the polypeptide.

Please replace paragraph [0148] beginning on page 39 with the following amended paragraph:

[0148] A modified GPCR containing a modified carboxyl-terminus region may be created by fusing a first carboxyl-terminal tail portion of a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin with a second carboxyl-terminal tail portion of a GPCR or polypeptide having one or more clusters of phosphorylation sites. The second GPCR or polypeptide used for the exchange may have a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif (SEQ. ID NO.: 82). Accordingly, the modified carboxyl-terminus region of the modified GPCR comprises a portion of a carboxyl-terminal tail from a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin fused to a portion of a carboxyl-terminal tail of a GPCR or polypeptide having clusters of phosphorylation sites. The tail of a GPCR lacking properly positioned clusters of phosphorylation sites may be exchanged after or including the conserved NPXXY motif (SEQ. ID NO.: 82), and fused to a carboxyl-terminal tail containing clusters of phosphorylation sites, after or including the conserved NPXXY motif (SEQ. ID NO.: 82). As an alternative, the tail of a GPCR lacking properly positioned clusters of phosphorylation sites may be exchanged after or including the palmitoylated cysteine(s), and fused to a tail containing clusters of phosphorylation sites, after or including the palmitoylated cysteine(s). The tails may be exchanged and the modified GPCRs may be constructed accordingly by manipulation of the nucleic acid sequence or the corresponding amino acid sequence.

Please replace paragraph [0149] beginning on page 40 with the following amended paragraph:

[0149] In a further alternative, the carboxyl-tail of a GPCR, for example a GPCR not containing the NPXXY motif (SEQ. ID NO.: 82), may be predicted from a hydrophobicity plot and exchanged accordingly. The site of exchange may be selected according to the hydrophobicity plot. Based on a hydrophobicity plot, one of skill in the art may predict a site where it is expected that the GPCR may anchor in the membrane and then predict where to introduce a putative site of palmitoylation accordingly. Using this techique GPCRs having neither a NPXXY motif (SEQ. ID NO.: 82) nor a putative site of palmitoylation may be modified to create a point of reference (e.g. a putative site of palmitoylation). The introduced putative site of palmitoylation may be then used to position a tail exchange. After introduction of a putative site of palmitoylation, the resulting tail may be fused with a second carboxyl-terminal tail portion of a GPCR or polypeptide having one or more clusters of phosphorylation sites and having a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif (SEQ. ID NO.: 82).

Please replace paragraph [0150] beginning on page 41 with the following amended paragraph:

[0150] Preferably, the modified carboxyl-terminus region of the modified GPCR is fused at amino acid residues in close proximity to a putative site of palmitoylation. More preferably, the modified carboxyl-terminus region of the modified GPCR is fused such that the portion from the first GPCR with a lower affinity for arrestin comprises amino acid residues from the NPXXY motif (SEQ. ID NO.: 82) through a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the NPXXY motif (SEQ. ID NO.: 82) and the portion from the second GPCR having clusters of phosphorylation sites and a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif (SEQ. ID NO.: 82) comprises amino acid residues beginning with an amino acid residue immediately downstream of the putative site of palmitoylation of the second GPCR extending to the end of the carboxyl-terminus. This fusion is preferred because the clusters of phosphorylation sites are reliably positioned properly within the carboxyl-terminal tail and the modified GPCR maintains its structure and ability to function.

Please replace paragraph [0264] beginning on page 76 with the following amended paragraph:

[0264] The nucleic acids of the GPCR of interest were PCR-amplified with primers that introduced a Not I restriction enzyme site (gcggccgc) immediately after the codon for a cysteine residue (a putative site of palmitoylation) 10 to 25 amino acids (preferably 15 to 20) downstream of the NPXXY (SEQ. ID NO.: 82) that is to be fused to the V2R carboxyl terminus. The amplified receptor DNA fragment was then subcloned into the pEArrB-1 vector using the Not I restriction enzyme site and an additional restriction enzyme site upstream of the receptor atg start codon. A schematic of the resulting pEArrB-1/GPCR vector is shown in Fig. 4B. When expressed, the modified GPCR will contain a 31 amino acid peptide fused to the receptor carboxyl terminus. The first two amino acids will be Ala residues contributed by the Not I site, and the last 29 amino acids will be from the V2R carboxyl terminus (see Fig. 4C).